Bioscience

A Metabolic Study of Biohydrogen-Producing Photosynthetic Bacteria: The Effects on Growth Rates of Rhodobacter capsulatus JP91 Hup- and Rhodopseudomonas palustris when Acetate is replaced by Glucose as the Primary Carbon Source

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The study of photosynthetic purple non-sulfur bacteria (PNSB) is a relatively new and encouraging field of biofuel research. These bacteria metabolize organic acids and, though less efficiently, sugars into hydrogen – a high-energy fuel source. If the efficiency of glucose-to-hydrogen conversion could be increased, many waste products could be directly converted into fuel. This project investigated relative growth performance of strains of PNSB when glucose replaced sodium acetate as the primary carbon source. The potential benefit of increased genetic diversity of mixed cultures was also explored. The growth rates of two bioreactors each containing *Rhodobacter capsulatus JP91 Hup-* were compared to those of two bioreactors populated with a consortium of PNSB (*R. capsulatus JP91 Hup-* and two strains of *Rhodopseudomonas palustris*). The bacterial cultures were grown anaerobically in constant flow photobioreactors. Growth rates were determined by measuring changes in biodensity. There was no significant difference in growth rates between monocultures and mixed cultures. However, the growth rates of bacteria on glucose were generally equal to or greater than those on acetate. This result suggests that further study of metabolic patterns of PNSB presented with various carbon sources may prove useful in exploring the viability of single-stage conversion of waste sugars into biohydrogen fuel.

L'étude de bactéries pourpres non sulfureuses (BPNS) est relativement nouvelle et un domaine prometteur à l'égard de la recherche sur les biocarburants. Ces bactéries métabolisent les acides organiques et, bien que de façon moins efficaces, métabolisent aussi les sucres à l'hydrogène – une source de carburant à haute énergie. Si l'efficacité de la conversion de glucose à hydrogène pouvait être augmentée, de nombreux déchets organiques pourraient être directement convertis en carburant. Ce projet a étudié le rendement de croissance relative des souches de BPNS lorsque le glucose fut remplacé par de l'acétate de sodium comme source de carbone primaire. L'avantage potentiel de l'augmentation de la diversité génétique de cultures mixtes a également été examiné. Le taux de croissance des deux bioréacteurs contenant chacun *Rhodobacter capsulatus JP91 Hup-* ont été comparées à deux bioréacteurs peuplés avec un consortium de BPNS (*R. capsulatus JP91 Hup-* et deux souches de *Rhodopseudomonas palustris*). Les cultures bactériennes ont été cultivées anaérobiquement dans des photobioréacteurs à débit constant. Les taux de croissance ont été déterminés en mesurant la variation de biodensité. Il n'y avait pas de différence significative dans le taux de croissance entre les monocultures et les cultures mixtes. Cependant, le taux de croissance avec le glucose était généralement égale ou supérieure comparé à l'acétate. Ce résultat suggère que d'autres études du profils métaboliques de PNSB avec diverses sources de carbones peuvent

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s'avérer utiles dans l'exploration de la viabilité de la conversion à étape simple de déchets organiques détenant du sucre en biohydrogène .

Introduction

A promising avenue in the research and development of renewable fuels is the production of hydrogen by photosynthetic bacteria. Photosynthetic bacteria can produce hydrogen in a way that is less energy-intensive than any other means[4]. These bacteria are abundant and can metabolize a wide range of organic compounds[3]. One example is purple non-sulfur bacteria (PNSB). Using PNSB to produce hydrogen offers the potential to utilise common waste products such as agricultural waste as a carbon source, providing both economic and environmental benefits. This will require a firm understanding of how PNSB respond to a range of carbon sources and how their metabolic efficiency can be increased. Although PNSB metabolism of glucose directly into hydrogen is not yet as efficient as the conversion of organic acids, it would be more useful and direct1. Currently, other bacterial systems (i.e. dark fermenters) must be used to first metabolize glucose into organic acids for use in PNSB systems[5]. This multistage process is more costly than an efficient single stage process would be.

This experiment explored the effects of periodically switching the main carbon source of PNSB from sodium acetate to glucose. Also explored was the potential benefit of increased genetic diversity. The growth rates of two bioreactors each containing only *Rhodobacter capsulatus JP91 Hup*$^-$ were compared to two bioreactors populated with a consortium of PNSB (*R. capsulatus JP91 Hup*$^-$ and two types of *Rhodopseudomonas palustris*). These three strains were selected because they are widely studied among current researchers.

The study of growth rates in addition to hydrogen production is an important area of research as it has been shown that changes in growth affect hydrogen production. While it is well established that hydrogen production is inhibited under high nitrogen conditions, a requirement for higher growth rates, it has also been demonstrated that hydrogen production is limited under resting state conditions. Consequently, achieving optimal hydrogen production requires a level of cellular growth[1]. This project was an investigation of relative growth performance of strains of PNSB when presented with glucose versus a short chain organic acid normally consumed by PNSB in vivo as a carbon source.

Given the ecological niche in which they have evolved, PNSB are not normally exposed to glucose as a primary carbon source; therefore, using glucose as opposed to organic acids as the main carbon source yields lower stoichiometric amounts of hydrogen[1]. On this basis, it was predicted that the substitution of glucose as a carbon source in place of sodium acetate would result in lower growth rates. It was hypothesized that the combination of *R. capsulatus JP91 Hup*$^-$ and two other strains of *R. palustris* would potentially lead to higher growth rates than a reactor containing only *R. capsulatus*, due to the increased culture diversity. It is generally accepted that increased diversity allows for a stronger, more adaptable system when under stress (stress in this case being the replacement of acetate with glucose).

Materials and Methods

Two cultures of *R. capsulatus JP91 Hup*$^-$ were grown in photobioreactors respectively labelled 1A and 2A while two mixed cultures containing *R capsulatus JP91 Hup*$^-$*, R. palustris CGA009* and *R. palustris DX-1* were grown in reactors 1B and 2B, respectively. The cultures were grown in anaerobic constant flow photobioreactors with a run-through rate of 50ml of nutrient per day per each 500ml reactor. The reactors and feed tanks were sparged with argon to maintain anaerobic conditions, and pH was monitored and maintained at 7.0. The reactors were incubated at a temperature of 30 degrees Celsius and illuminated with incandescent tungsten bulbs placed 15 cm above the reactors. The cultures were mixed with a magnetic stir bar. The bacteria were grown with a modified basal medium with added thiamin and iron. The carbon and nitrogen sources were added directly to the reactors during the course of the experimental runs. The acetate carbon source was periodically replaced with glucose. Acetate was obtained by titrating acetic acid with sodium hydroxide. Monosodium glutamate was used as the nitrogen source. Four con-

secutive one-week experimental runs for each reactor are reported. The carbon and nitrogen sources in millimolar concentrations for each run were as follows:

Run 1: acetate (20mM) glutamate (10mM)
Run 2: glucose (6.7mM) glutamate (10mM)
Run 3: acetate (20mM) glutamate (10mM)
Run 4: glucose (6.7mM) glutamate (10mM)

*Note that the total mM of carbon remained at 90mM for each run (this includes the carbon derived from the glutamate). The ratio of carbon to nitrogen remained constant at 9:1- a ratio generally used for promoting cell growth.

Biodensity was measured every 4-12 hours using a calibrated turbidity meter. The data was plotted and transposed into dry weight per volume by weighing dried centrifuged samples of each of the cultures.

Results

Figure 1 provides a summary of the calculated results in Table 1. In nearly all of the experimental runs conducted for all four reactors, the growth rates of the bacteria grown with glucose equaled or exceeded the growth rates of the bacteria with acetate. For example: within reactor 1A (a monoculture) during the first set, the growth during the glucose run phase was 152% of the growth of that culture during the acetate run. During the second set, the compared growth rates were similar. In Figure 2, it can be seen that the growth rates for mono and mixed cultures (for both the acetate and glucose mediums) were similar within the range of experimental error. There is no statistically significant increase in performance for more diverse reactors. Table 2 provides specific values.

Reactor – Culture Type	Set 1 (Run 2/ Run 1)	Set 2 (Run 4/ Run 3)
1A - Mono	152%	102%
1B - Mixed	96.6%	132%
2A - Mono	128%	108%
2B - Mixed	202%	126%

Table 1. Summary of the relative growth rates of cultures grown on glucose compared to those grown on acetate. Values of 100% indicate that growth rates on glucose were the same as on acetate. Values of over 100% indicate higher growth on glucose than acetate. This table corresponds to the summary presented graphically in Figure 1.

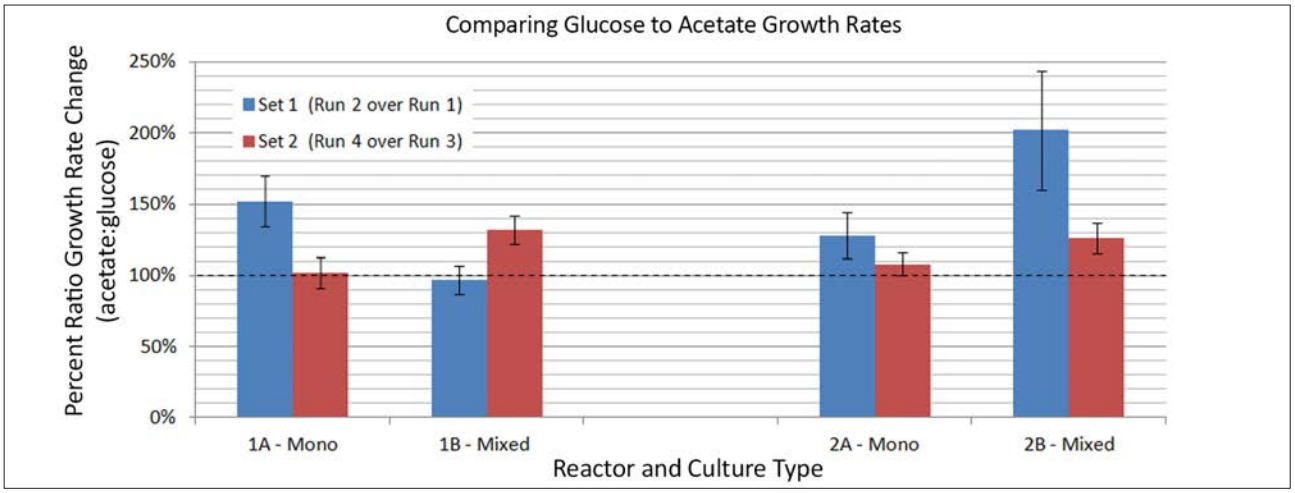

Figure 1. In nearly all runs, the growth rates of the bacteria grown with glucose equaled or exceeded the growth rates of the bacteria with acetate. The percent increase of growth per day was calculated for each reactor (1A, 1B, 2A, 2B). Within each data set (where a data set includes an acetate experimental run and a glucose experimental run), the difference in growth rates of the acetate run and the glucose run was calculated by dividing the glucose growth rates by the acetate growth rates. The 100% line on the vertical axis represents the level at which the growth rate during the glucose run equals the growth rate during the acetate run. Thus, where the bar extends above the 100% mark, growth was higher during the glucose run than during the acetate run. Set 1 represents run 1 (the first glucose run) compared to run 2 (the first acetate run). Set 2 represents run 3 (the second glucose run) compared to run 4 (the second acetate run). Refer to the Materials and Methods section for detail on runs.

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Carbon Source	Culture Type	Average Growth per Day
Acetate	Mono	28.7%
Acetate	Mixed	30.9%
Glucose	Mono	34.7%
Glucose	Mixed	38.5%

Table 2. The increase in average growth per day of mixed cultures versus monocultures was within the range of the estimated experimental error; also represented in Figure 2.

Abbreviation

PNSB Purple non-sulfur bacteria
ml Millilitre
cm Centimeter
mM Millimolar

Key Words

Anaerobic; waste; glucose; purple non-sulfur bacteria; biofuel

References

[1] Abo-Hashesh, M.; Ghosh, D.; Tourigny, A.; Taous, A.; Hallenbeck, P. C. Single Stage Photofermentative Hydrogen Production From Glucose: An Attractive Alternative to Two Stage Photofermentation or Co-Culture Approaches. Int. J. Hydrogen. Energ. online, 2011, 36, 13889- 13895. doi: 10.1016 /j.ijhydene.2011.02.122

[2] Abo-Hashesh, M., Desaunay, N., Hallenbeck, P.C., High yield single stage conversion of glucose to hydrogen by photofermentation with continuous cultures of Rhodobacter capsulatus JP91, Bioresource Technology, online, 2012, 128, 513-517. doi: 10.1016/j.biortech.2012.10.091.

[3] Mckinlay, J. B., Harwood, C. S., Harnessing Bacteria That Use Light to Produce Hydrogen, Microbe, online, 2011, 6, 345-351.

[4] Androga, D. D., Biological Hydrogen Production On Acetate In Continuous Panel Photobioreactors Using Rhodobacter Capsulatus, Energy and Environment, online, 2009, 78-2

[5] Keskin, T., Abo-Hashesh, M., Hallenbeck, P. C., Photofermentative Hydrogen Production from Wastes, Bioresource Technology, online, 2011, 102- 18, 8557- 8568, doi: 10.1016/j.biortech.2011.04.004

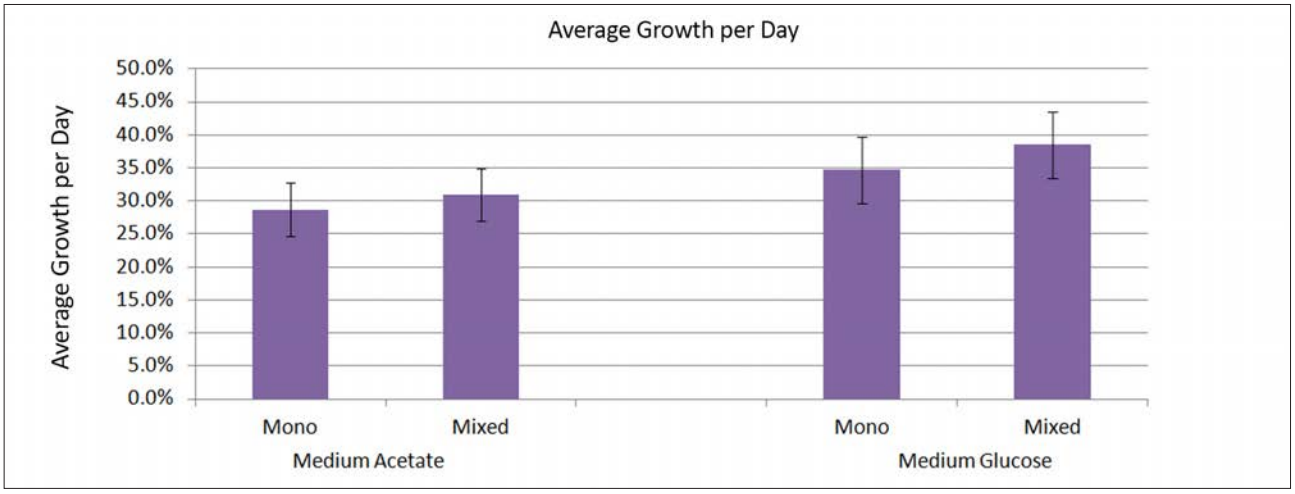

Figure 2. There was no statistically significant change in performance for reactors with greater culture diversity. Figure 2 compares the relative growth rates of monocultures vs. mixed cultures for both carbon sources. The average of the growth in reactors 1A and 2A was used to reflect average monoculture growth. The 1B and 2B growth rates were averaged to determine mixed culture growth rates. See Materials and Methods section for detail on reactor content.

Review of ***A Metabolic Study of Biohydrogen-Producing Photosynthetic Bacteria:*** *The Effects on Growth Rates of Rhodobacter capsulatus JP91 Hup- and Rhodopseudomonas palustris when Acetate is replaced by Glucose as the Primary Carbon Source*

In this study, Ms. Preston demonstrates how varying a carbon source can influence the growth characteristics of R. cap in a mono- and a mixed culture. She effectively illustrates, using tables and figures, these photosynthetic anaerobes increased their growth on glucose rather than the more traditional carbon source, acetate, when measured using a turbidity meter.

In the context of significant scientific merit towards to real-world problems this research is second-to-none. These types of studies are currently being done in large research labs globally. The liquid biofuels industry is screening hundreds of compounds including carbon sources like the ones used in Ms. Preston's study. These metabolic assays are also being coupled with altering other abiotic factors such as light (quality and quantity), temperature, and ionic strength.

Ms. Preston's study is carefully done as she tightly controls many variables within the photobioreactors so that she is certain that the carbon source is the only cause of differential growth characteristics between the mono- and mixed cultures. She has clearly made a considerable effort to identify controls and design her experiments attentively. This is very impressive. Some scientists take years to be good at this.

In several respects this research paper is very well written and organized. The figures and tables are clear and help direct the reader through the data set that could have become complicated without a thorough description. I do feel that the captions for figure 1 and table 1 are lengthy and these long descriptions should be left in the text of the paper rather than in the captions. In all other ways this paper is really a homerun. Her abstract is concise and appropriate in length. And, her use of the primary literature exceeds my expectations for a student at this level. It was my pleasure to review Ms. Preston's exceptional manuscript.

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